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Critical Review

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Quantities of marine debris ingested by sea turtles: Global meta-analysis highlights need for standardized data reporting methods and reveals relative risk

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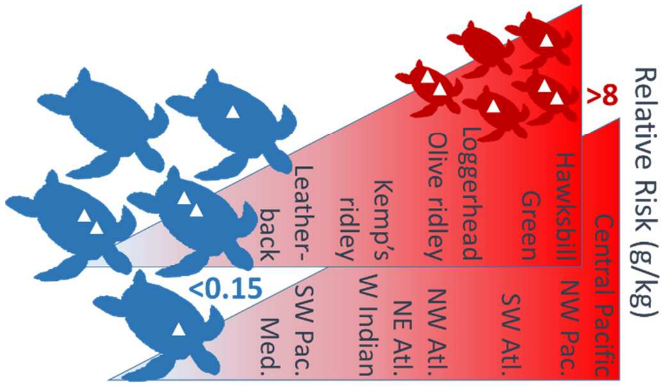
Abstract

Because of their propensity to ingest debris, sea turtles are excellent bioindicators of the global marine debris problem. This review covers five decades of research on debris ingestion in sea turtles from 131 studies with a novel focus on quantities. Previous reviews have focused solely on presence/absence data. Past reviews have called for standardization and highlight biases in the literature, yet none thoroughly describe improvements needed at the data reporting stage. Consequences of three reporting choices are discussed: not reporting quantities of ingested debris (32% of sea turtle studies reported only frequency of occurrence), excluding animals that did not ingest debris (64%), and not normalizing quantities to animal size (95%). Ingestion quantities, corrected for these factors, allowed a first-ever global meta-analysis on the units of g/kg, revealing that hawksbill and green turtles rank highest among sea turtle species, and that the Central and Northwest Pacific and Southwest Atlantic Oceans are hotspots. Furthermore, this review discovered that monitoring efforts are disproportionate to the magnitude of the problem. Large efforts are focused in the Mediterranean Sea where international policies are hotly discussed versus the Central Pacific that has 5-fold greater debris ingestion quantities but represents only 3 % of the global research effort. Future studies are recommended to report quantities of ingested debris using units described herein and make use of the pilot database provided.

Keywords: anthropogenic debris, plastic ingestion, marine turtles, standardization, risk assessment

TOC Art


Relative Risk (g/kg)
>8
<0.15
Central Pacific
Hawksbill
Green
Loggerhead
Olive ridley
Kemp's ridley
Leather-back
NW Pac.
SW Atl.
NW Atl.
NE Atl.
W Indian
SW Pac.
Med.

INTRODUCTION

Marine plastic debris is considered an emerging contaminant and requires vigilant monitoring [1, 2]. For sea turtle populations, debris ingestion is a concerning and increasing anthropogenic threat that deserves more detailed scientific study [3-9]. A global model estimated that 52 % of surviving sea turtles have likely ingested marine debris [10] and a reported 4 % of necropsied sea turtles have died from it [11]. Because of their propensity to mistake debris for their natural prey, sea turtles, like seabirds, are recognized as excellent bioindicators for monitoring the amounts and types of marine debris in a region [12, 13].

Standardizing methods to facilitate spatial, temporal, and species comparisons are critical. As Koelmans et al. [14] pointed out, "*present measurement methods are far from fully developed or standardized among laboratories*." This problem is particularly evident in the five decades of research on debris ingestion in sea turtles. Recently the European Commission has published recommended methods for sea turtles [15], and additional international groups are currently working to standardize methods in marine debris research. Plastic litter is one of the few classes of contaminants that can be seen with the naked eye, making isolating mega, meso, and 1 mm to 5 mm microplastics from sea turtle gastrointestinal (GI) tract contents relatively straightforward. Although painstaking work, it only requires simple laboratory methods, at most microscopy, rather than sophisticated and expensive chemical instrumentation. Despite simple methods, researchers could greatly improve methods at many stages of analysis: turtle collection, debris collection, and data analysis and reporting.

The first two stages (turtle and debris collection) have been thoroughly discussed in other reviews with excellent recommendations for sea turtle studies [11, 16]. Casale et al. [16] is an authoritative review of numerous flaws or biases in 49 sea turtle studies. At the turtle collection stage, the sources of turtles (e.g., stranded vs. picked alive) each have biases and comparing studies using these different sampling strategies presents challenges [16]. Stranded turtles do not represent the normal sea turtle population in a given region [16]. Turtles foraging in pelagic regions have a greater likelihood of debris ingestion than neritic benthic feeding stages [10, 16]. Therefore, ingestion data should be reported in a stratified manner for different sources of turtles, size classes (or pelagic vs. neritic phases), fishing gear used, stranding locations (beach vs. at sea), and body conditions, especially if injury or illness modified foraging [16]. Sample sizes are routinely low in opportunistic studies on protected species, and this unfortunate disadvantage makes certain kinds of debris ingestion data, like frequency of occurrence, "*essentially meaningless*" [16].

At the debris collection stage, assessing the entire GI tract during necropsy is considered the most effective sampling strategy compared to lavage or fecal assessments [11, 16-19]. Method choices for identifying debris items (e.g., with or without microscope, different sieve mesh sizes or digestion/extraction methods) likely affect debris detection among gut contents and bias the size range of items discovered. Additionally, studies with alternative goals (e.g., diet, mortality causes, or other pollutant types) likely underestimate debris ingestion, and the quality of data differs between these types of studies so comparisons are affected [16]. Few studies provide clear descriptions and counts of lethal cases of debris ingestion, but all should [16].

Additional scrutiny and improvements are needed at the data analysis and reporting stage. No marine debris ingestion review study has discussed all aspects of this stage, but Provencher et al. [20] provided several recommendations. The goals of the current review were to thoroughly discuss biases at only this stage and perform the first global review of marine debris ingestion in sea turtles focused on quantities rather than presence/absence. Frequency of occurrence (%FO), or the proportion of turtles assessed that contained ingested debris, has been the focus of all previous review articles on debris ingestion in sea turtles [11, 16, 21, 22]. The consequences of reporting only %FO and two other data reporting choices (excluding non-detects and failing to scale debris amounts to turtle size) are presented. After correcting for non-detects and normalizing to turtle size, average ingestion quantities (g/kg) were estimated from previous studies for a more appropriate assessment of geographical hot spots and species of concern. Advantages of recommended units are provided for future studies to consider.

STRATEGY OF REVIEW

References that investigated plastic marine debris ingestion in sea turtles were gathered from previous comprehensive global or regional literature reviews [11, 12, 16, 19, 21-25] and from Google Scholar searches for studies published between 2014 and April 2018 using the search terms: “marine debris ingestion sea turtle” and “plastic ingestion sea turtle”. Non-peer-reviewed reports were included. Studies were categorized based on data reporting choices. If quantities were reported, studies were categorized based on units reported and whether non-detects were included in calculating the averages. Non-detects are turtles without observed ingested debris.

Turtle body size measurements and plastic ingestion data were entered into a spreadsheet (pilot database). To maximize comparability among studies and make the best use of previously published data from diverse units, numerous estimations converted carapace length to kg and plastic ingestion quantities to grams of debris using science-based conversions or experienced judgement. The most frequently used conversions are explained below, all equations can be found in the database (Supporting Information File S1). The unfortunate conversions were necessary to maximize the number of studies and make the best use of previously published data from diverse units. The goal was to obtain an entry into the spreadsheet for as many studies as possible for three data columns: 1) average mass of ingested debris per turtle, 2) average kg of turtles, and 3) average g debris/average kg of turtle.

When non-detects were not included in averages of ingested plastic quantities, the average quantities were accurately recalculated using the following equations:

(average quantity without non-detects x number of detections) / total number of turtles assessed

or

total quantity of debris measured in entire study / total number of turtles assessed.

Since the debris ingestion quantity for non-detects is somewhere between zero and the detection limit, statisticians do not recommend substituting their value with a zero [26]. However, in this meta-analysis, zero substitution was necessary and is justified based on the size of debris. Nearly all studies targeted micro- to meso-plastics (>1 mm [20]), which are hard to overlook when assessing sea turtle gut contents. Detection limits and appropriate handling of non-detects will become more important in future studies focusing on smaller debris sizes.

Reported debris quantities in pieces/turtle or mL/turtle were converted to grams/turtle with equations from data in Clukey et al. [19] [g = (0.11885*pieces) + 0.9289 or g = (0.5964*mL) + 0.9817], being cautious at the high and low ends of the range (e.g., 1 piece cannot be converted accurately to grams). These regressions are based on 2880 plastic debris items ingested by 50 pelagic-phase Pacific sea turtles representing three species. Regressions within studies to convert number of pieces to g/turtle for individual tabulated turtles [27] or ratios of max pieces/turtle to max g/turtle within a study were used on occasion [28]. The total mass of debris in all turtles within a study could be calculated by multiplying the reported % of gut contents that was debris by the total gut contents mass in all turtles (occasionally both were reported). This total debris quantity divided by the number of turtles assessed provided an accurate average g/turtle. On several occasions, studies did not provide debris mass, but did provide length and width dimensions of all debris items ingested, or these could be estimated from photos. Surface area of each ingested debris item was calculated using the following equations and required the assumption that each piece was a box shape,

$$\mathbf{cm^2 \text{ of each piece} = 2 \times [\, (L \times W) + (L \times D) + (W \times D) \,]}$$

where L is length, W is width, and D is depth in cm, and

total cm^2 per turtle = sum of surface area of all pieces in one turtle.

When D was not provided, it was assumed to be 1 mm for hard fragments and 0.5 mm for sheets; W and D for pieces of line were both assumed to be 0.5 mm. These were average values of common items measured in Clukey et al. [19]. Then, the estimated surface area of each debris piece was converted to mass using data collected by Jung et al. [29]. That study had recorded the dimensions, surface area, and mass of >800 individual plastic debris items ingested by 50 pelagic-phase Pacific sea turtles (representing three species). By matching the type, dimensions, and surface area, the mass of pieces from other studies could be estimated. In rare cases, new items of similar type and size were weighed to represent the debris mass (plastic fork, straw, and bags) found within a turtle [22, 30-32].

When turtle mass (kg) was not reported, it was estimated using carapace length (cm). Curved carapace length (CCL) was converted to straight carapace length (SCL) using equations for each species [33]. Likewise, SCL was converted to mass using equations for each hard-shelled species [33]. For leatherback turtles, CCL was converted to mass [34, 35]. When only the ranges of turtle size rather than a mean was reported [17], the mean kg was estimated from other studies with similar ranges of the same species.

For debris quantities in units of g/kg, two columns of data were included in the database: 1) actual reported g/kg and 2) a calculation of average g of debris divided by average kg of turtle within the study. In rare cases, both g ingested and kg of turtle mass was available from all individual turtles, allowing the actual values (g/kg) to be tabulated per turtle [19, 36]. For the second column, the numerator, denominator or both could be estimates.

The database was then sorted by species and geographical regions representing roughly quadrants of ocean basins. When a turtle was reported in multiple publications, data were selected so that each turtle was represented only once within a region. Studies focused solely on cause of death (e.g., Meager and Limpus [37]) were excluded from the meta-analysis, since turtles that ingested debris but were not killed by it are not be represented in these data. Data from remaining studies within a region for a particular species were combined to calculate average %FO and debris quantities weighted by sample size within that region.

Selection criteria to potentially exclude studies or samples based on turtle or debris collection methods were carefully considered for the meta-analysis. An overly inclusive approach was ultimately chosen to maximize the number of regions available for comparison and sample sizes within regions at the risk of some known disadvantages. Turtle collection years were from 1950 to 2017, even though debris ingestion has increased during this time [38]. Studies with diverse turtle sources were included, but when possible, turtles with empty GI tracts due to reduced foraging caused by illness (e.g., 30 turtles in Matiddi et al. [13]) were excluded as recommended by Casale et al. [16]. No effort was made to select pelagic- versus neritic-phase age classes, even though pelagic-phase turtles ingest debris more frequently [10, 11, 16, 22, 28, 39-41].

Studies containing any method of debris collection (fecal, lavage, portion of gut, and entire gut assessments) were included, but when one study provided data from two methods, the most comprehensive data were chosen (e.g., gut rather than fecal data [42]). Attempts were made to select data on only synthetic debris (e.g., only plastic [43, 44]), but some studies only reported a combined quantity for natural and synthetic debris. Natural debris (rocks, plant matter, feathers, etc) is common, but plastic is frequently the majority of ingested debris. Excluding studies for any reason described above would have eliminated or halved sample sizes in certain regions. The coarse assessment made herein was intended to provide relative rankings of regions or species rather than a statistical assessment of significant differences.

A brief discussion of the limitations and benefits of this overly inclusive approach is necessary. Combining studies with diverse methods increases the variability within a region or species, especially when only two studies with very different methods are combined from one region. Readers are encouraged to carefully consider the data and methods within each resulting data point. All turtles from a region were combined for one weighted average. Because data on individual turtles was rarely available, no variance could be calculated for each weighted average. It was also not possible to calculated medians, which are preferred over averages in contaminant studies because data are typically log-normal and skewed to the right. Benefits to this approach are that the meta-analysis includes non-detects, maximizes the use of all sea turtle ingestion studies, even from incredibly diverse units, and weights regional and species averages to total sample size, which allows for inclusion of single-turtle case studies in an appropriate and meaningful way. Furthermore, it provides average quantity estimates for each reviewed study to facilitate future comparisons if reporting recommendations herein are adopted. The strength of this manuscript is the critical review requesting better data for better quantitative comparisons in the future. The current approach is the best use of poor data to demonstrate gaps and point out critical needs.

SUMMARY OF REVIEWED STUDIES

From 1970 to April 2018, 131 publications were found that assessed plastic ingestion by sea turtles. The field grew linearly until 2007, after which it has been rising exponentially (Figure 1). The database lists all studies by species, shows method categorization, tabulates results, and provides calculations for estimating turtle sizes and debris ingestion quantities (Supporting Information File S1).

Sample sizes ranged from 1 to 777 turtles/study with 27 % of the studies containing only a single turtle (Figure 2). Ninety-three percent of the studies observed debris ingestion (Figure 3). This is likely an overestimation because studies that did not find debris are less likely to be published [21]. When debris was observed, 114 studies (93.4 %) reported %FO while only 83 (68.0 %) reported a debris quantity with a diversity of units (Figure 3). Of these 83 studies, 62 (74.7 %) included a sample size greater than one. Even after five decades of research, discussion of method standardization has been very limited and only recent. Furthermore, no discussion has focused on standardizing reporting methods until now.

CONSEQUENCE OF DATA REPORTING CHOICES

Excluding non-detects. Of the 62 studies that reported a quantity and had a sample size >1, only 35.5 % (22 studies) intentionally included non-detects in their calculation of central tendency (Figure 3). Excluding non-detects can greatly overestimate a population's mean ingestion amount. For example, using data from Hoarau et al. [45], loggerheads that ate plastic ingested 41 items on average, but when the 36 additional non-detects are included, the average drops to 19 items/turtle (Supporting Information File S2 Figure S1). Excluding non-detects overestimated the average plastic ingestion of this Western Indian Ocean sea turtle aggregation by 22 items/turtle or 2.2 times (116 %). This is most important when comparing different populations or aggregations. To demonstrate this consequence, grams of debris ingested by green turtles at three locations along Brazil are compared both ways (Figure 4). Excluding non-detects from Paraíba mistakenly depicts it as ranking second with 2.3-times more mass ingested than turtles from Santos. When considering all turtles studied, Paraíba turtles rank lowest and actually ate 2.9-times less than those near Santos. Both examples show how excluding non-detects can be misleading.

The decision to include or exclude non-detects is often overlooked or under-explained. Provencher et al. [20] discusses this issue. Their recommendation, and mine, is to include non-detects. Statisticians also plea that non-detects should not be excluded in calculating central tendency and variance, or when testing hypotheses with statistics [26]. These individuals are equally important to a population's average ingestion quantity as an individual that ate debris. For these reasons, all quantities shown in Supporting Information File S2 Table S1 are corrected to include non-detects.

In one situation, certain non-detects might best be excluded. Casale et al. [16] points out that turtles with empty GI tracts, such as chronically ill, emaciated stranded turtles, are biased and do not represent the general turtle population. When neither plastic nor food is observed in a turtle GI tract, another stressor must have reduced foraging behavior for several weeks prior to death. Without intentional foraging, these turtles could not have mistaken plastic for food; thus, researchers should consider excluding these turtles. On a broader scale, Nelms et al. [21] pleas for studies to publish both positive and negative results. If debris was not observed in the gut of any turtles, these delightfully "negative" findings deserve to be reported so they can be included in comparisons.

Frequency of occurrence versus quantities. Of the 122 studies that found debris ingestion, only 8 studies (or 6.6 %) did not report a %FO whereas 39 (or 32.0 %) failed to report an ingestion quantity. Because %FO is the most commonly reported metric of debris ingestion, it has been the sole focus for previous sea turtle review articles [11, 12, 16, 21-25]. Provencher et al. [20] noted that %FO is the most commonly reported value in seabird studies, so the sea turtle literature is not alone in this disadvantage.

Because %FO does not describe how much debris was ingested, it is not as valuable as describing the quantity ingested. It is possible for two studies to find very high %FO but substantially different ingestion quantities. For example, two studies with similar methods assessing plastic mass in the entire GI tract of juvenile green sea turtles, one from Central Pacific [19] and one from Brazil [46], had similarly high %FO of ≥90 % (Figure 5). However, the quantity ingested in the Central Pacific green turtles was 7-times greater than the Brazilian turtles. This comparison suggests that the risk of adverse effects would be higher in Central Pacific turtles and that presence/absence data (%FO) is not enough to explain risk.

Diversity of units. Units for reporting quantity of debris ingested per turtle varied widely across the 83 studies (Figure 3 inset). The most common unit (71.1 % of studies reporting a quantity) was number of debris pieces, followed by debris mass (41.0 %). Six of the latter studies reported wet debris mass, which overestimates the quantity ingested. For example, the dry debris mass ingested by 64 Pacific sea turtles was on average 24 % less than the wet mass (22 % SD) (Lynch, unpublished data). Debris volume (18.1 %) and surface area (20.5 %) per turtle were occasionally reported or enough data were provided so these could be estimated. In 24.1 % of the studies, authors reported a percentage of gut contents, but the methods are diverse, including wet masses, dry masses, or volumes, and sometimes the denominator is the entire gut contents, while other times it is isolated prey items. Furthermore, the method sections of these papers often lack sufficient definitions of the numerator and the denominator, causing confusion and difficulty when comparing data across laboratories. Only four studies (4.8 %) provide enough data to calculate grams of debris per kg of individual turtle, and one of these assessed only one turtle. The low percentage of studies reporting quantities, even just counts of debris pieces, is disappointing and the diversity of units makes it challenging to compare across space, time, species, life stages, etc. Nelms et al. [21] and Clukey et al. [19] also called for sea turtle studies to standardize methods for reporting quantities so that comparisons can be made across available studies.

UNIT RECOMMENDATIONS

Each unit of quantity has its advantages and disadvantages, including biasing interpretation of results and methodological challenges (Supporting Information File S2 Tables S2-3). Of the units listed on a per turtle basis, debris dry mass has been recommended [19, 21]. Advantages of this unit include a good measure of body burden, a moderate number of studies in the existing literature have reported this unit for comparison, it correlates to the number of debris pieces, surface area, and volume per turtle [19], and it avoids overestimations caused by fragmentation of pieces [47] (Table S2). Reporting the number of pieces/turtle is also warranted, even though its major disadvantage is that larger pieces encountered by a turtle may be fragmented in the mouth or gut during ingestion or digestion [21] or during handling of the sample. Although time consuming, measuring each piece on all three sides for surface area calculations and for reporting the longest dimension is also helpful. Knowing the size distribution of debris provides more information about selection of debris by species. Surface area might be the best unit for assessing transfer of additive or sorbed chemicals from plastic to turtles, although debris mass should work well too.

Toxicologists often paraphrase Paracelsus by saying "*the dose makes the poison*." Conventional toxicity thresholds are stated on a mass fraction basis, such as g of pollutant per kg body mass. One toxicity threshold has been proposed for plastic ingestion in sea turtles. Santos et al. [48] concluded that 0.5 g of ingested plastic could kill green sea turtles, but this value was not put in context of the mass of the turtles they studied near Brazil. This very low threshold is puzzling, because pelagic turtles in the central Pacific frequently contain 20 g or more of debris in their GI tracts yet show no sign of adverse effect (e.g., no blockage, perforation, ulcer, or malnutrition) [19, 49]. The risk of health impacts from ingesting 0.5 g of plastic is presumably higher for a 6.5 kg green turtle (37 cm long) than a 29 kg green turtle (60 cm long), even though both are still considered juvenile, let alone a 290 kg adult leatherback (135 cm long). The same is true for comparisons among different sized fish, seabird, or marine mammal species. As vertebrates grow in length and mass, so does the diameter of their GI tract. Therefore, it is reasonable to expect a higher risk of gut obstructions in smaller compared to larger sea turtles [21, 28]. This speculation is most concerning given the consistent result that smaller, pelagic sea turtles are more likely to ingest debris than larger, older, benthic stages [10, 11, 16, 22, 28, 39, 40]. Normalizing debris ingestion units to the size of the turtle is therefore important, especially if the goal is to compare younger to older age classes or across different species that have different mature body sizes. On the other hand, it is critical to note that size does not always matter when it comes to effects of ingested plastics. Many studies have noted that small amounts can cause health problems for sea turtles [11, 17, 48, 50]. For instance, a small sharp item could perforate the gut of a large turtle causing sepsis and death, or a single fishing line can cause plication of intestines [51]. Regardless, documenting the amount ingested per turtle size is ideal for comparing across studies, species, life stages, space, and time and for generally assessing risk of harm.

Therefore, debris mass per turtle mass (g/kg) is by far the optimal unit for reporting marine debris ingestion quantities. Weighing turtles is logistically difficult (Table S3), which explains why only 21 of the 83 studies that reported ingestion quantities also reported a mean turtle mass compared to 54 reporting a mean CCL. Turtle measurements are important and mass data are worth the extra effort. Turtle mass data can also be used in the calculation of body condition index which should be used by more sea turtle studies [16, 19]. The best studies will report their data using multiple units with a focus on pieces/turtle, debris dry mass/turtle, debris dry mass/kg, and surface area/turtle (Table S3), while also making good use of Supporting Information files to provide raw data on each individual turtle. Size dimensions of debris items, required to calculate surface area, are important to report too. Volume is a more uncertain measurement than mass, and % of gut contents is too complicated, so they are less favorable. If a global database is maintained for monitoring marine debris ingestion by sea turtles, as called for recently [11, 21], data entry fields for quantities and turtle size will encourage future studies to collect and report on recommended units.

META-ANALYSIS COMPARISONS

Particular debris ingestion quantities from the database were extracted into a summary table (Supporting Information File S2 Table S1). This table provides corrected quantities of ingested marine debris in recommended units for all 131 studies and shows which data were used for the meta-analysis. Species and geographical comparisons could be made for the first time on quantities of ingested plastic. Figure 6 provides the outcome of the meta-analysis for a) research effort, as measured by the percentage of all turtles assessed, b) %FO, and c-f) multiple units of quantities ingested by green sea turtles across geography. Figure 7 provides the same for loggerhead sea turtles. Supporting Information File S2 contains Figures S2-S5 for the additional species. Finally, species and geographical comparisons using the preferred unit, g/kg, are mapped globally in Figure 8.

Findings by species. Effort of assessing debris ingestion has varied greatly across species or regions. Regardless of location, green and loggerhead turtles have received the most attention with 2795 and 2250 individuals assessed, respectively. Totals for other species were 621 leatherbacks, 444 Kemp's ridleys, 88 olive ridleys, 82 hawksbills, and at least 4 flatbacks.

Green sea turtle. Great effort has gone into studying green turtles in Brazil and not so much elsewhere (Figure 6a), even though green turtles in other locations, such as the Central and NW Pacific, ingest higher quantities (Figures 6d-f). %FO typically ranges from 0 to 88% and is >50% in the Central Pacific, NW Pacific, NW Atlantic, and SW Atlantic. Regions with the lowest %FO are the Mediterranean Sea, E Tropical Pacific, or E Indian Ocean.

Examining quantities, Central Pacific green turtles ate >70 pieces/turtle, similar to turtles in the SW Atlantic, which is >100-times more than those in the Gulf of Mexico, Mediterranean Sea, SW Pacific, or E Tropical Pacific (Figure 6c). By inspecting grams/turtle, the NW Pacific green turtles ate the most, followed by Central Pacific green turtles, which ate 2.2-times more than SW Atlantic greens and >100-times more than greens in the Gulf of Mexico and SW Pacific (Figure 6d). Comparing these quantities to the 0.5 g/turtle threshold proposed by Santos et al. [48], the average green turtle in the NW Pacific exceeds this critical value 44-fold, 20-fold in Central Pacific, 9.3-fold in SW Atlantic, and 4.4-fold in NW Atlantic. Average green turtles elsewhere are below this threshold (Figure 6d).

By scaling the ingestion quantities to body size, differences between post-hatchlings (9 cm) and adults (120 cm) are normalized and can be compared (Figure 6e-f). Using the units g/kg, Central Pacific turtles ate about two-times more than greens from the SW or NW Atlantic Ocean and >100-times greater quantities than Gulf of Mexico or SW Pacific Ocean (Figure 6e). NW Pacific greens ate double that of Central Pacific greens. It was surprising that SW Atlantic greens were not higher than Central Pacific, because Brazilian researchers frequently report death from debris ingestion while no pathology is observed in the Central Pacific turtle GI tracts. Clukey et al. [19] suggested that the difference may be due to smaller turtles assessed in Brazil. However, this meta-analysis does not support that possible explanation (Figure 6e). The reasons for the differences in effect remains to be determined. Differences could be due to source of turtles (stranded in Brazil vs. healthy Pacific live captures) or differing interpretation of GI pathology. Regardless, scaling the quantities ingested to body size is important when making these comparisons. The threshold proposed by Santos et al. [48] (0.5 g/turtle) was converted to 0.077 g/kg using the estimated average body mass of turtles assessed in that study (6.48 kg). Average turtles in four regions are above this threshold (dashed line in Figure 6e). Turtles in the Central Pacific exceeded this value by 12-times without observed effect, suggesting that this threshold value is not accurate for all green turtle populations and needs further refinement to be used globally.

To remove the possible bias of emaciation from stranded animals, spatial differences were investigated in g/cm (Figure 6f). SCL will not fluctuate with weight gain or loss, so it could possibly be a better measure for scaling to body size. This analysis shows the same trends as g/kg with Central Pacific green turtles eating ≈2- to 4-times more than greens from the SW Atlantic or NW Atlantic and >100-times more than the Gulf of Mexico or SW Pacific. NW

Pacific greens ate twice as much as Central Pacific greens. Using these units (arguably a better choice for the comparison of stranded in Brazil vs. bycatch in Central Pacific), ingestion quantities still do not explain the difference in death attributed to debris ingestion.

Loggerhead sea turtle. Fifty-three studies have assessed plastic ingestion in loggerhead sea turtles across 12 global regions with more than half of the effort focused in the Mediterranean Sea followed by the NW Atlantic Ocean (Figure 7a). The heavy effort in the Mediterranean is likely driven by policy and subsequent research funding. Besides National Action Plans in each Mediterranean country, marine debris is managed by two regional entities: the UN Environment/Mediterranean Action Plan (Regional Plan on Marine Litter Management in the Mediterranean) and the European Commission (Marine Strategy Framework Directive), and the loggerhead turtle is a target indicator species for monitoring the marine debris problem [12]. To my knowledge, there is no other policy directive or reduction goal for other sea turtle species or regions of the world even though the environmental issue is worse for other species and regions. Loggerhead %FO typically ranges from 0 to 90% globally (Figure 7b). Regions with >50 %FO are the NW Pacific and NE Atlantic. The N Central Pacific ranks 5th among regions with 45 %FO, while the Mediterranean ranks 7th with 40 %FO. No debris was observed in neritic-phase loggerhead turtles inhabiting the E Tropical Pacific and E Indian Ocean.

A comparison of Figure 7b and 7c further demonstrates that %FO may be misleading because it tells a different story than quantities. For example, N Central Pacific loggerheads had a lower %FO at 41.6% compared to 61.7 % of NE Atlantic loggerheads, but the N Central Pacific turtles ate 7.6-times more pieces/turtle than NE Atlantic turtles (Figure 7c). In fact, the N Central Pacific loggerhead turtles ate many times more pieces than any other region with an average of 83 pieces/turtle (Figure 7c). W Indian and NE Atlantic loggerheads rank 2nd and 3rd

with about 10 pieces/turtle. The Mediterranean Sea ranks 4^{th} with 5 pieces/turtle, which further demonstrates that research effort is proportional to policy not the magnitude of the problem. Even lesser amounts were consumed by loggerheads in the SW Atlantic, Gulf of Mexico, and NW Atlantic. The NW Pacific suffers from a low sample size for the unit of pieces/turtle. By inspecting grams/turtle, a better geographical comparison can be made, the NW Pacific turtles ate the most, twice as much as the N Central Pacific loggerheads, followed by W Indian, Gulf of Mexico, NE Atlantic, and Mediterranean Sea turtles (Figure 7d).

On a g/kg basis, W Indian loggerheads consumed the most (0.47 g/kg), likely because they were post-hatchling turtles, followed by N Central Pacific (0.26 g/kg), NW Pacific (0.18 g/kg), NE Atlantic (0.17 g/kg), and Mediterranean Sea (0.05 g/kg) (Figure 7e). The regional rankings in g/cm were NW Pacific>N Central Pacific>W Indian>NE Atlantic>Gulf of Mexico>Mediterranean (Figure 7f). The top three switched ranks from those of g/kg, but this comparison fails to support the expected effect of weight loss on debris ingestion quantities. The NW Pacific turtles were dead stranded, so they were expected to consist of some emaciated turtles. Their body condition (thus denominator in g/kg) was likely lower than those of the N Central Pacific turtles that were all fisheries bycatch in good body condition. A small denominator would increase g/kg in the NW Pacific relative to the N Central Pacific, but the opposite was observed. This comparison begins to suggest that g/kg is equally as good as g/cm.

Leatherback sea turtles. Thirty-one studies have assessed plastic ingestion in leatherback turtles across 11 global regions. Half of the effort is in the NW Atlantic, with most of the remaining effort split equally between the NE Atlantic and off Peru's coast around 1980 (Figure S2a). Leatherback %FO ranges from 12.5 to 55% globally, excluding regions with only a single turtle assessed (Figure S2b). The Mediterranean and NE Atlantic Ocean rank the highest in %FO. An assessment of quantity becomes difficult because of low sample sizes within many regions (Figures S2c-f). Confidently, we can observe that Central Pacific and NW Atlantic leatherbacks eat 4-5 pieces/turtle; 1-2 pieces/turtle in W Tropical Atlantic and NE Atlantic, and none in SW Pacific (Figure S2c). W Tropical Atlantic (French Guiana and Brazil) appears as the highest-ranking region for g/turtle (Figure S2d), but this is greatly skewed by one turtle that died from gut blockages caused by 2.6 kg of debris. On a g/kg basis, W Tropical Atlantic and W Indian leatherbacks rank highest with the former value skewed by the one turtle (Figure S2e). Looking at g/cm (curved carapace length), W Indian leatherbacks rank the highest, while the W Tropical Atlantic value becomes very small because the length of the turtle that ate 2.6 kg of debris was not measured (Figure S2f). The removal of this one individual from the sample set explains the discrepancy between g/kg and g/cm.

Kemp's ridley sea turtles. Eleven studies have assessed plastic ingestion in Kemp's ridley turtles across three regions. Effort has been concentrated in the Gulf of Mexico (78%), followed by NW Atlantic (20%) and NE Atlantic (2%) (Figure S3a). %FO is generally lower than other species and ranges from 0% in NE Atlantic to 25% in the Gulf of Mexico (Figure S3b). Quantities regardless of unit were highest in Gulf of Mexico Kemp's ridleys (Figures S3c-f).

Olive ridley sea turtles. Seven studies have assessed plastic ingestion in olive ridley turtles across three regions. Most of the effort has been in the Central Pacific with less than 5% of effort in SW Atlantic and E Tropical Pacific (Figure S4a). %FO ranged from 75% to 100% (Figure S4b). Quantities in pieces/turtle and g/turtle are $\geq$ 4-times more in Central Pacific ridleys than SW Atlantic and E Tropical Pacific (Figures S4c-d). When scaled to turtle size, quantities ingested were greater in the Central Pacific than SW Atlantic (Figure S4e-f).

Hawksbill sea turtles. Eleven studies have assessed plastic ingestion in hawksbill turtles across seven regions. Most of the effort has been split between SW Atlantic, Caribbean/Gulf of Mexico, and SW Pacific, with less than 5% of effort in the following regions: NW Atlantic, NE Atlantic, Central Pacific, and E Tropical Pacific (Figure S5a). %FO ranges from 8.3% in SW Pacific to 100% in the NW Atlantic and Central Pacific, excluding regions with N = 1 (Figure S5b). An assessment of quantity becomes incredibly difficult because of low sample sizes within many regions (Figures S5c-f). Confidently, we observe that SW Atlantic hawksbills ingested 12 pieces/turtle compared to NW Atlantic hawksbills eating 2.5 pieces/turtle (Figure S5c). On a g/turtle basis, Central Pacific hawksbills rank highest with the two individuals eating an average of 39 g/turtle, which is skewed by one turtle with a gut blockage of debris mixed with feces that weighed 780 g (debris mass was estimated at 10% or 78 g) (Figure S5d). In SW Atlantic hawksbills, 1.1 g/turtle was observed. On a g/kg basis, Central Pacific ranks the highest at 8.8 g/kg (potentially skewed high by the outlier), compared to 0.73 g/kg in 13 SW Atlantic hawksbills (Figure S5e). The same ranking was seen on a g/cm basis (Figure S5f).

Flatback sea turtles. Three studies assessed debris ingestion in flatback sea turtles, all of which are from Australia. Two studies contained only a single turtle each. One turtle was found with threads of plastic debris in its esophagus that were part of a larger mass that had entangled the turtle. It is not clear if the turtle intentionally ingested the debris [52]. Another flatback turtle was reported in Schuyler et al. [53] as ingesting marine debris. Finally, less than six flatback turtles that stranded between 1998 and 2011 were assessed for cause of death and at least one of them died from ingestion of debris [37]. None of these studies reported quantities so graphs like the other species could not be generated.

GLOBAL COMPARISON AND DATA GAPS ACROSS SPECIES

Figure 8 maps the average debris ingestion for six species of sea turtles on units of mean g/mean kg. Hawksbill sea turtles have strikingly high quantities compared to other species in the same region. This is true for the SW Atlantic, NE Atlantic, Central Pacific and Eastern Tropical Pacific regions. This finding emphasizes the urgent need to monitor the threat of plastic ingestion in hawksbill sea turtles.

Green turtles often ingest larger quantities than loggerheads, olive ridleys, or leatherbacks within a region. Leatherback and Kemp's ridley turtles consistently ingest the lowest quantities. An obvious data gap is the lack of quantities reported for the relatively abundant green sea turtles from the Mediterranean Sea. While the loggerhead sea turtle is proposed as the best indicator species for monitoring marine litter for policy targets [12], green turtles eat more debris than loggerheads in other regions. Therefore, this data gap should be addressed in the Mediterranean Sea.

Previous studies have attempted species comparisons using only %FO, and they largely disagreed with each other. Balazs [22] concluded the following trend in likelihood to ingest debris: green>loggerhead>leatherback>hawksbill. The data included at this early stage of research was biased towards only detections; non-detects were certainly excluded which likely skewed the trend. Schuyler et al. [11] presented the following trend which differed substantially from Balazs [22]: hawksbill>green>leatherback>(loggerhead=Kemp's ridley). Finally, an updated trend resulting from a global modelling exercise provided the following trend: olive ridley>(green=loggerhead=hawksbill=leatherback)>Kemp's ridley [10]. Of these, the Schuyler et al. [11] trend aligns the best with the current meta-analysis using debris ingestion quantities scaled to turtle size: hawksbill>green>(loggerhead=olive ridley)>Kemp's ridley>leatherback.

Additional studies on all species are needed, but an increase in effort for hawksbill sea turtles is imperative. This species is classified as "Critically Endangered" by the International Union for Conservation of Nature's Red List because many of its populations have been declining [54]. Plastic ingestion has caused the death of several hawksbills [22, 36, 37], and they appear to ingest far greater quantities than other species (Figure 8). Despite these good reasons for focused effort, only 82 individuals across only seven regions globally have been assessed. Schuyler et al. [10] highlighted hawksbills, as well as leatherbacks, as species with data gaps. Because pelagic-phase turtles ingest larger quantities than neritic-phase turtles, a directed increased effort to assess younger pelagic-phase turtles of all species is warranted, as previously recommended by Nelms et al. [21].

GLOBAL COMPARISON AND DATA GAPS ACROSS GEOGRAPHY

Turtles in the Pacific Ocean, especially the NW, Central, and E Tropical Pacific regions, ingest much higher quantities than elsewhere. The SW Atlantic comes in a close second. Turtles in the Mediterranean Sea and coasts along the continental U.S. and Australia have the lowest ingestion concentrations. These rankings may change as new data are reported. For example, since completion of this meta-analysis, two studies have been published [55, 56]. Post-hatchlings of three species stranded along Florida's east coast were reported to ingest on average 2.07 g/kg [55]. This new study would likely increase the symbol sizes in Figure 8 for loggerhead, green, and hawksbill turtles in the NW Atlantic. Fourteen green turtles can now be added to the NW Indian Ocean region with an estimate of 0.03 g/kg [56], which would result in the empty green circle in Figure 8 becoming shaded.

The map in Figure 8 for green, loggerhead and olive ridley sea turtles is congruent with higher concentrations of sea surface debris in the pelagic gyres, especially in the Pacific and

South Atlantic Oceans [57], but is complicated by different sources of turtles in different locations. The known bias of greater ingestion in pelagic-phase turtles compared to neritic-phase turtles, and the inclusive approach that lumped both together for this analysis, could be a confounding factor in the geographical comparisons.

This geographical comparison can begin to ground truth the global risk analysis performed two years ago before several studies reviewed here were available [10]. Their model (Fig 3 therein; note the Mediterranean was not included) predicted the highest risk for loggerheads in the NE Indian Ocean, followed by equal risk in the N Pacific, Gulf of Mexico, Caribbean Sea, and NW Atlantic to Portugal. Regions of lesser risk were predicted in the W Indian Ocean, near the Azores, followed by the SW Atlantic, S Pacific, and Australia. The current meta-analysis of actual ingestion data on a g/kg basis (Figure 7e or 8) shows the following ranking: W Indian Ocean>N Central Pacific>NW Pacific>NE Atlantic>Gulf of Mexico>NW Atlantic>SW Pacific=E Tropical Pacific. There are several similarities but two major differences. Turtles monitored in the W Indian Ocean ingested far more than predicted, and turtles in the NE Indian Ocean have yet to be assessed for ingestion. For green turtles, Schuyler et al. [10] predicted the following geographical trends: South China Sea>(NE Indian Ocean=NW Pacific=W Indian Ocean)>NW Atlantic including the Gulf of Mexico and Caribbean Sea>nearshore Hawaii>(E Indian Ocean=W Africa=SW Atlantic=Australia)>(S Central Pacific=E Tropical Pacific=Central Atlantic). The current review found the following trend: NW Pacific>Central Pacific>SW Atlantic>NW Atlantic>E Tropical Pacific>SW Pacific>Gulf of Mexico/Caribbean Sea>NW and E Indian Ocean. The predictions missed the second hottest spot by not including much of the North Pacific pelagic realm in their model and greatly underestimated risk in S Central Pacific and SW Atlantic. Finally, olive ridley sea turtles in the

SW Atlantic and E Pacific were predicted to have similar risk, and slightly greater risk was predicted for ridleys in the W Pacific [10]. Actual ingestion data suggest that the risk for olive ridleys in the E Pacific (sampling occurred mainly in the N Central Pacific) is much higher than those in the SW Atlantic. Future risk analyses should focus on using quantities ingested rather than %FO.

In 2015, a review article concluded that most studies had been performed in the Atlantic Ocean [21]. When assessing effort to date by the number of turtles assessed for ingested debris quantities (g/kg basis), the majority of effort has occurred in the SW Atlantic Ocean (36%), mostly along Brazil's coastline, followed by the Mediterranean Sea (19%). The following regions have between 5% and 10% of the total turtles assessed: Gulf of Mexico/Caribbean Sea, Central Pacific, N and NW Indian, NW and NE Atlantic Oceans. Regions with <5% of the total effort include E Tropical Pacific, SW Pacific, NW Pacific, E Indian, and NE Pacific. Regions that have no published ingested quantities include NE Indian, SE Pacific, and SE Atlantic Ocean (excluding the southern point of South Africa).

Regions of overlapping sea turtle habitat and high marine debris concentrations in the surface waters should receive the highest priority for future research. Based on a map of concentrations published by Cozar et al. [57], effort should be increased in the pelagic zones between 25 and 35 degrees N, and between 25 and 35 degrees S. This is especially true for the NE Pacific, SE Pacific, N Central Atlantic, S Central Atlantic, and W Indian Oceans. Obtaining samples of pelagic-phase sea turtles is difficult, but the extra effort is required to fill these data gaps. Greatly underrepresented coastal regions that have a high likelihood of large inputs of debris include the Caribbean Sea, coastlines of Africa, NE Indian Ocean, South China Sea, and the Pacific coast of South America. These regions are nearly devoid of sea turtle plastic ingestion data.

CONCLUSION

With the importance and increasing magnitude of the marine debris threat to sea turtles, it is apparent that standardization at all stages of study is needed in this important field of research. Recommendations in prior reviews should be heeded for the animal and debris collection stages [11, 16, 21] as well as at the stage of data reporting [20]. The current review explained the consequences of failing to include non-detects, focusing solely on %FO rather than quantities, and neglecting to scale quantities to the size of animals. Studies are therefore recommended to report %FO, pieces/animal, debris dry mass/animal, and g debris/kg of animal. Sizes of debris items, at least the longest dimension, should also be reported, and surface area/animal can be useful too. Until now sea turtle review articles have focused solely on %FO, which depicts an incomplete story without quantities. Quantities, and especially g/kg, most accurately reveal species differences and hot spots of marine debris ingestion, emphasizing hawksbill and green turtles as the species at most risk and the Central and NW Pacific and SW Atlantic Ocean regions as the worst hot spots identified to date. Solutions to this environmental crisis, policies, and research funding are desperately needed for the most at-risk species, in the most problematic regions, and in regions that have yet to be monitored that may prove to be even worse (e.g., NE Indian Ocean). The database (Files S1) and averages of g/kg estimated herein for each reviewed study (Table S1) can serve as a basis for future comparisons. The described consequences and recommendations for data reporting may be equally important for taxa other than sea turtles.

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Disclaimer. Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Supporting Information Available. The pilot database of ingested marine debris quantities from 131 studies is File S1; File S2 contains tables listing advantages, disadvantages, and recommended debris quantity units, extracted from the database used for the meta-analysis, and meta-analysis results for four sea turtle species. This information is available free of charge via the Internet at http://pubs.acs.org.

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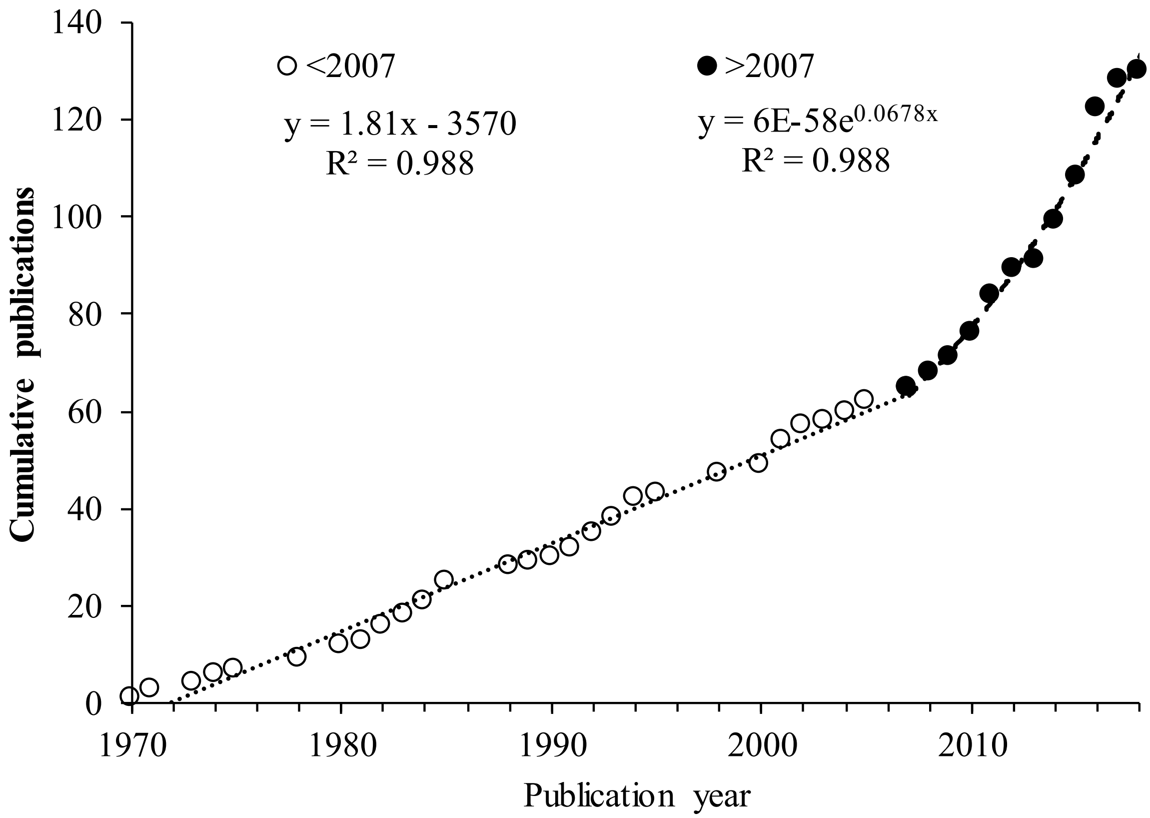


Figure 1. Cumulative number of publications increase linearly in the field of marine debris ingestion in sea turtles, becoming exponential after 2007 (excludes publications that were strictly review articles).

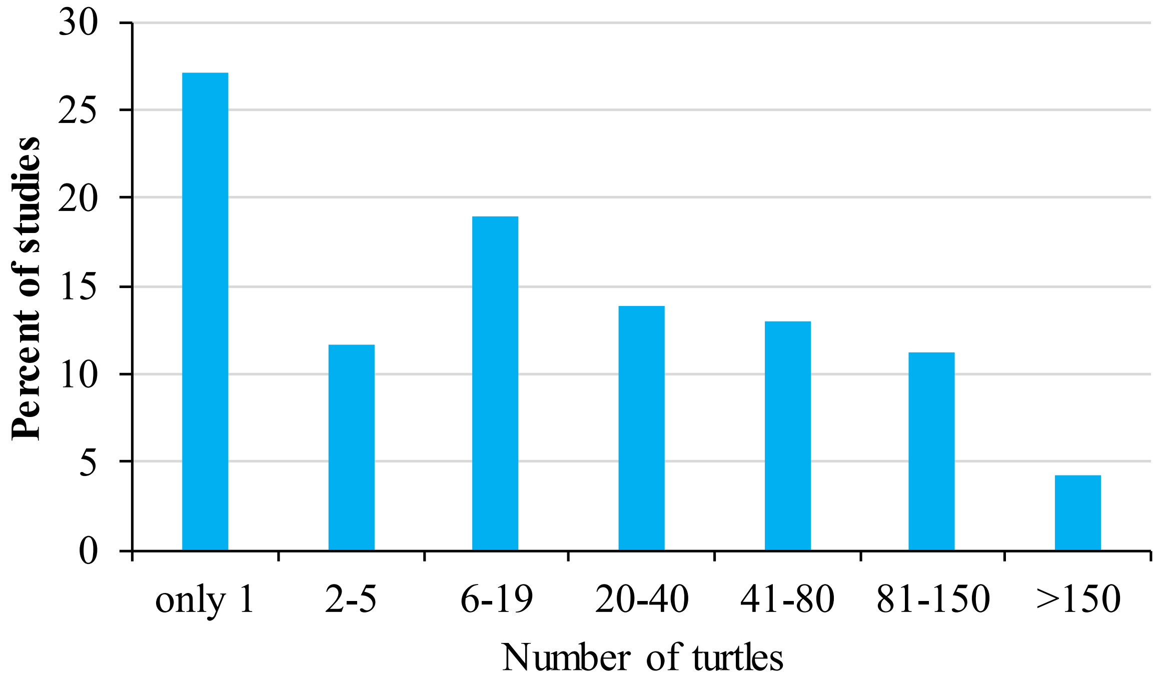


Figure 2. Sample sizes in studies assessing debris ingestion in sea turtles. Studies that contained more than one species were counted multiple times, once for each species.

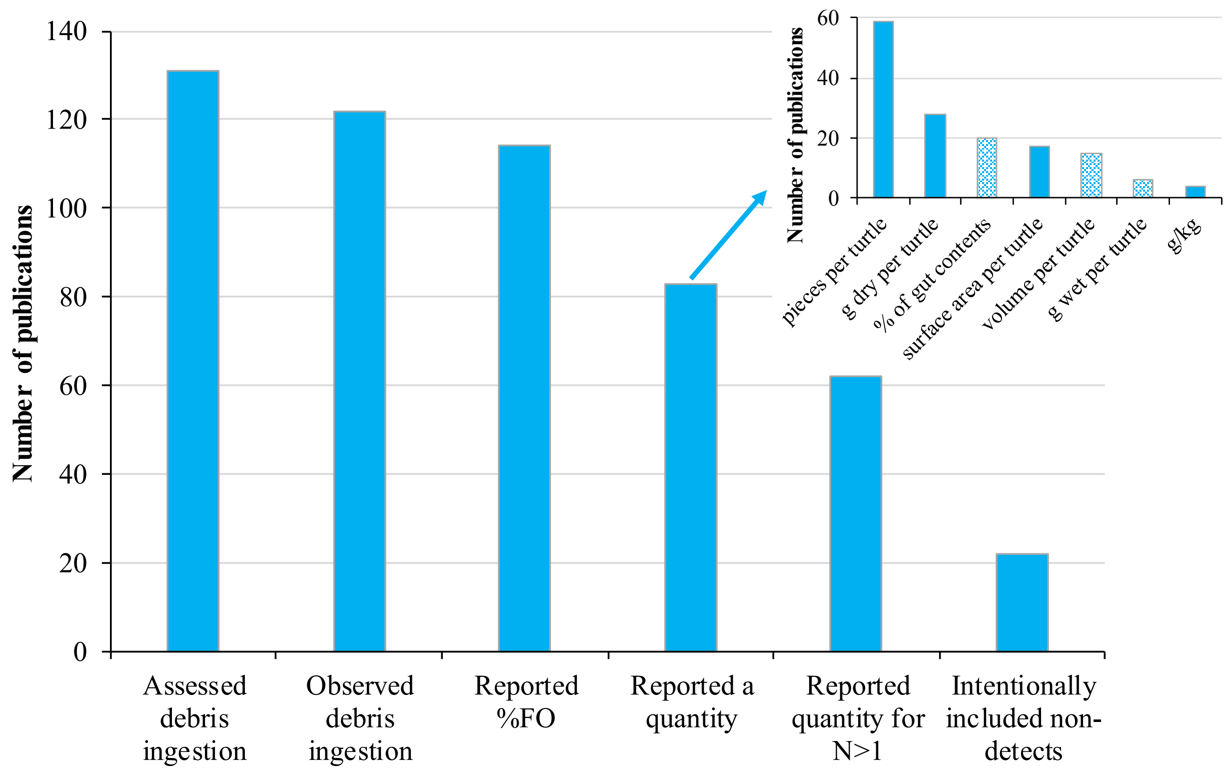


Figure 3. Reporting choices in studies assessing debris ingestion in sea turtles. Studies containing more than one species were counted only once for this assessment. Patterned bars indicate methods that are not recommended herein.

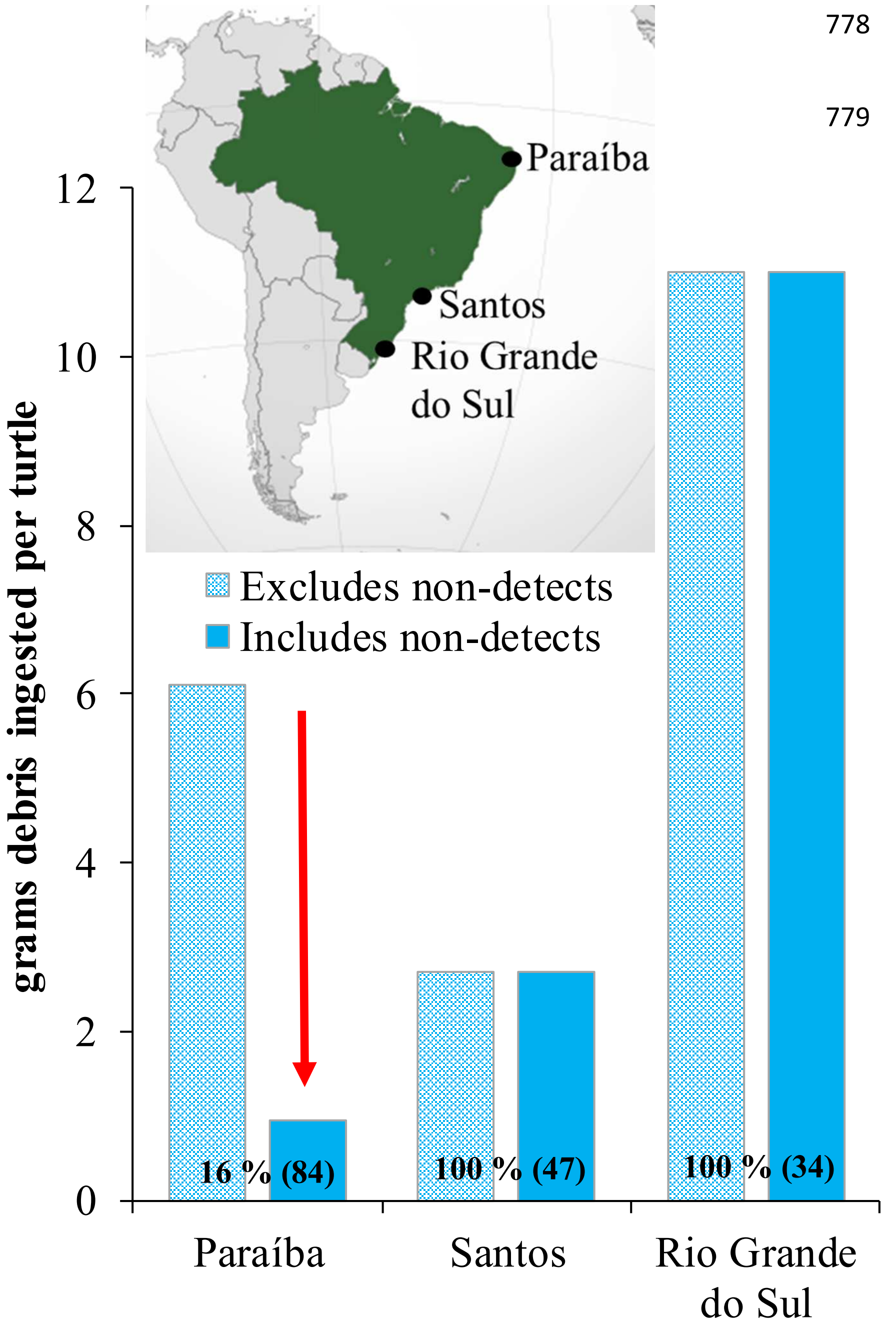
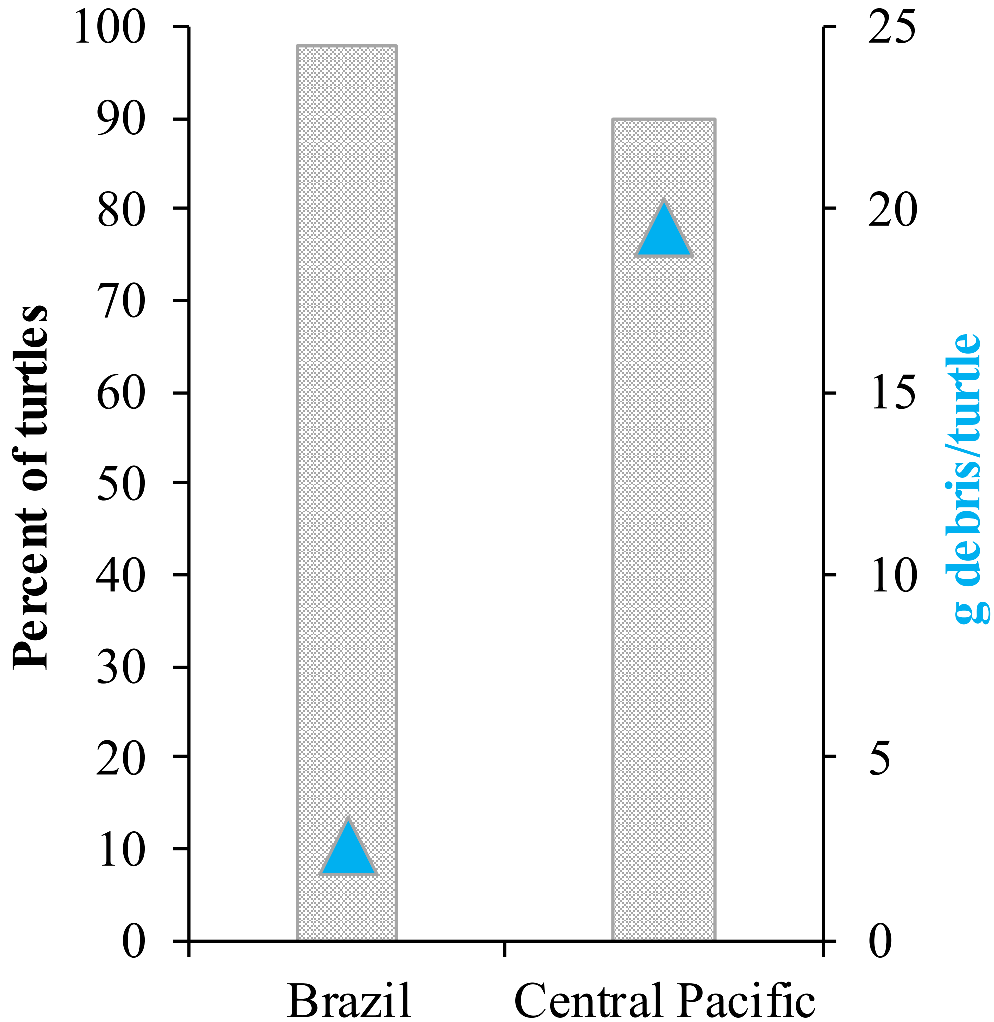


Figure 4. Comparison of excluding or including non-detects when reporting ingested debris quantities. Graph depicts average ingested debris masses from green sea turtles stranded at three locations along the Brazilian coastline [36, 46, 58]. Percent frequency of occurrence (sample size) are shown within bars. Patterned bars indicate methods that are not recommended herein.



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798 Figure 5. Example of percent frequency of occurrence (bars) versus quantity (blue triangle) of
799 ingested debris in green sea turtles from two locations [19, 46].

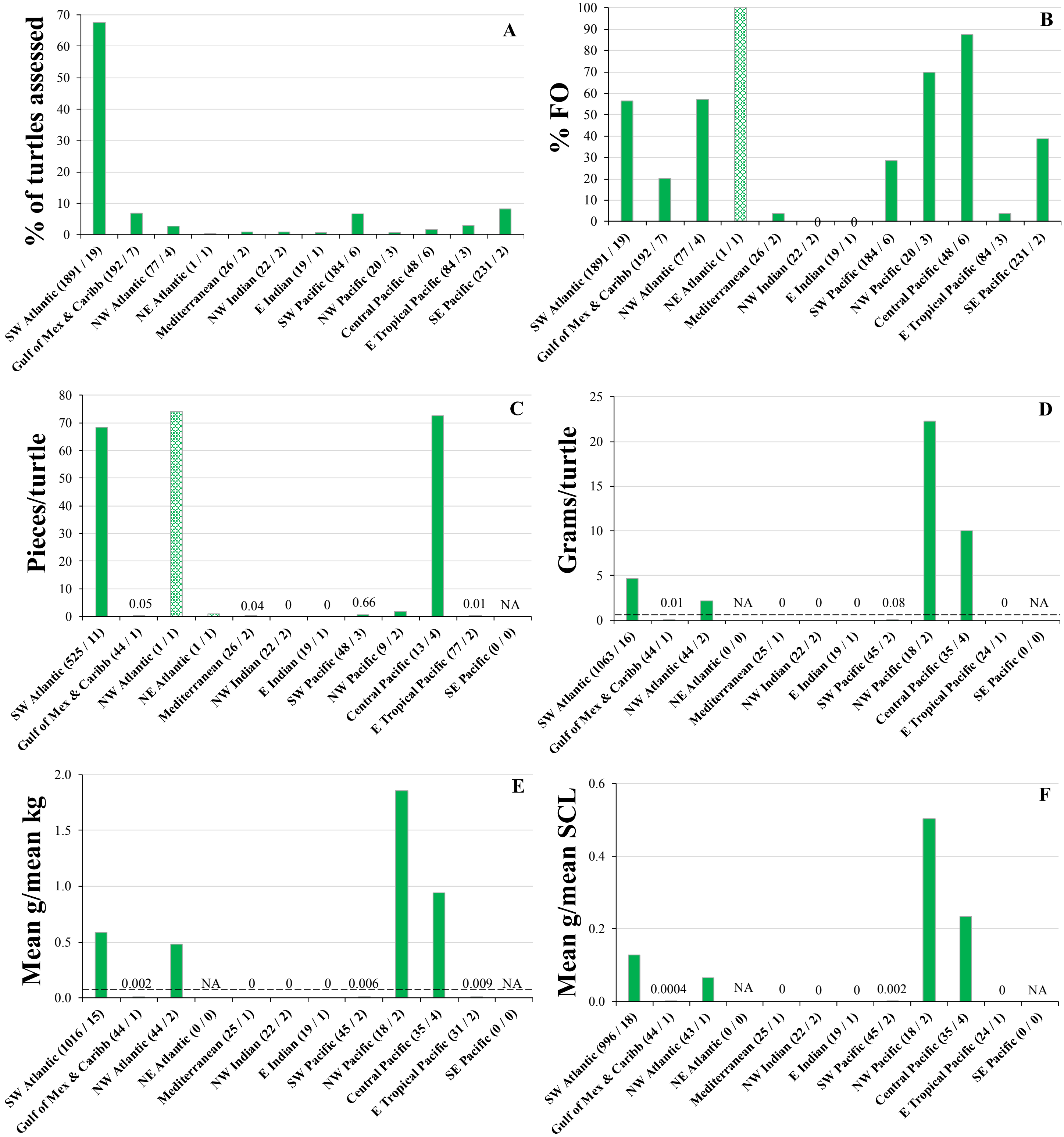

Figure 6. Geographical meta-analysis of green sea turtle ingested marine debris using different units: a.) % of turtles assessed, b.) % frequency of occurrence (%FO), c.) average number of pieces ingested per turtle, d.) grams of debris ingested per turtle (estimated or reported), e.) mean grams of debris ingested per mean kg of turtle (numerator or denominator could be estimated or reported), and f.) mean grams of debris ingested per cm

of straight carapace length (SCL) of turtle (numerator or denominator could be estimated or reported). Graphs c through f are weighted averages; variance could not be calculated; the intent is only to rank the regions. Patterned bars indicate only a single turtle and should be compared with great hesitation. Numbers above bars are mean values, zeros indicate no ingestion, and NA indicates that no data were available. Values inside parentheses indicate (number of turtles assessed / number of studies) within a region. Dashed lines are thresholds proposed by Santos et al. [48] (0.5 g/turtle) or the threshold divided by the converted average kg body mass of turtles assessed therein.

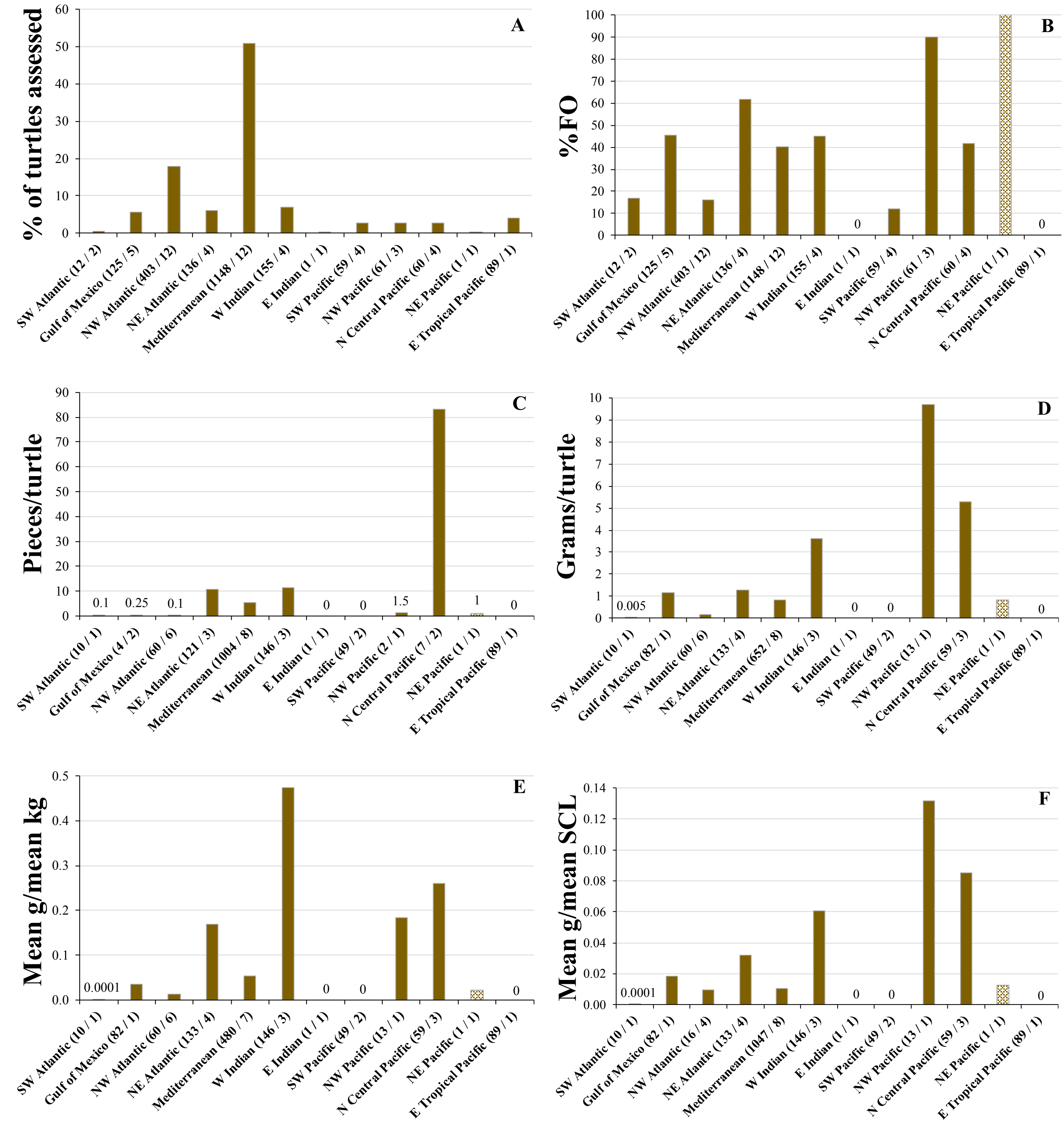

Figure 7. Geographical meta-analysis of loggerhead sea turtle ingested marine debris using different units: a.) % of turtles assessed, b.) % frequency of occurrence (%FO), c.) average number of pieces ingested per turtle, d.) grams of debris ingested per turtle (estimated or reported), e.) mean grams of debris ingested per mean kg of turtle (numerator or denominator could be estimated or reported), and f.) mean grams of debris ingested per cm

of straight carapace length (SCL) of turtle (numerator or denominator could be estimated or reported). Graphs c through f are weighted averages; variance could not be calculated; the intent is only to rank the regions. Patterned bars indicate only a single turtle and should be compared with great hesitation. Numbers above bars are mean values, zeros indicate no ingestion, and NA indicates that no data were available. Values inside parentheses indicate (number of turtles assessed / number of studies) within a region.

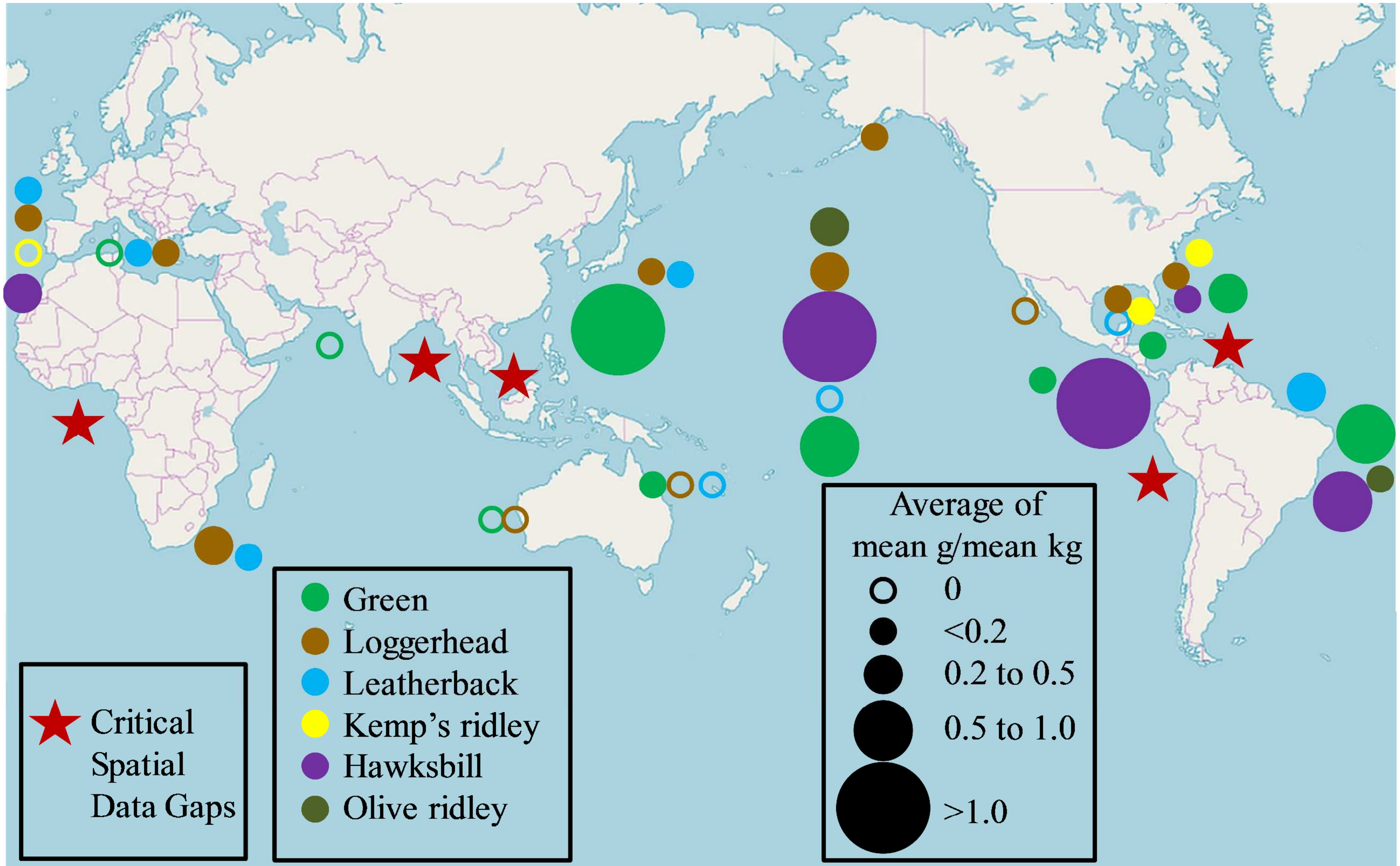

Figure 8. Global map of average mean g/mean kg of debris ingestion quantities in six sea turtle species. Different colors represent different species. Different sizes of circles indicate the quantity range. Data compiled from studies noted in Supporting Information File S2 Table S1.